

U.S.S.N. 08/970,045
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AMENDMENT

with the specific lipoprotein in a conformation and lipid content independent manner;

allowing the monoclonal antibody molecules to bind to the apolipoproteins in the sample,

immersing into the mixture third immobilized monoclonal antibody molecules immunoreactive with a second, distinct epitope of one of the first or second apolipoproteins,

allowing the third immobilized monoclonal antibody molecules to bind to one of the apolipoproteins bound by either the first or second monoclonal antibodies,

determining the amount of apolipoprotein bound by the first and second monoclonal antibodies and the amount of protein bound by the third immobilized monoclonal antibodies, and

[subtracting from] determining the difference between the total apolipoprotein bound by the first [and second] monoclonal antibodies and the amount of protein bound by the third immobilized monoclonal antibodies, to yield the [amounts] amount of the first [and second apolipoproteins] apolipoprotein and determining the difference between the total apolipoprotein bound by the second monoclonal antibodies and the amount of protein bound by the third immobilized monoclonal antibodies, to yield the amount of the second apolipoprotein.

40. (three times amended) A method for determining the relative ratio of VLDL to HDL in a biological sample comprising

(a) determining the amount of VLDL in the sample by

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determining the amount of Apo C-III present in the VLDL in the sample by
providing Pan B antibody which is characterized by an equal binding and
high affinity for all Apo B-containing lipoproteins in human plasma,
providing monoclonal antibody specifically immunoreactive with Apo C-III,
contacting the anti-ApoC-III antibody reactive with Apo C-III with the
biological sample to form complexes between the anti-ApoC-III antibody and the
Apo C-III containing lipoprotein particles,

contacting the Pan B antibody with the biological sample containing the anti-
ApoC-III antibody bound to the Apo C-III containing lipoprotein particles to form a
complex of the Pan B antibody with the anti-ApoC-III antibody-lipoprotein
particles,

separating the complexed Pan B-anti-ApoC-III antibody-lipoprotein particles
from the biological sample, and

determining the amount of complexed Pan B-anti-ApoC-III antibody-
lipoprotein particles, which is the amount of Apo C-III present in VLDL in the anti-
Apo C-III anti-Apo B complexed material in the sample;

and

(b) determining the amount of HDL in the sample by

determining the amount of Apo C-III present in the HDL in the sample by
providing Apo A-I monoclonal antibody specifically immunoreactive with Apo
A-I,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

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contacting the antibody reactive with Apo C-III with the biological sample to form complexes between the anti-Apo C-III antibody and the Apo C-III containing lipoprotein particles,

contacting the anti-Apo A-I antibody with the biological sample to form complexes with the anti-Apo C-III antibody-Apo C-III containing lipoprotein particles,

separating the complexed anti-Apo C-III antibody-Apo C-III containing lipoprotein particles from the biological sample,

determining the amount of Apo C-III present in HDL in the anti-Apo C-III-anti-Apo A-I complexed material in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample to Apo C-III present in HDL in the sample, which is the ratio of VLDL to HDL,

wherein the VLDL and HDL are measured in the same sample using immobilized anti-Apo A-I and anti-Apo B or anti-Apo C-III antibodies or measured by immunoprecipitation with the anti-Apo A-I and anti-ApoB antibodies or anti-Apo C-III antibodies in separate samples,

wherein at least one of the monoclonal antibodies bind to a stable, conformation independent epitope that is uninfluenced by the lipid content of the lipoprotein, apolipoprotein or lipid associated with a specific lipoprotein selected from the group consisting of Apo AI, Apo B, and Apo CIII.

44. (three times amended) A kit for determining the relative ratio of VLDL to HDL comprising